THE ACTION OF LEUKOCYTIC EXTRACTS ON THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES

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A large amount of work has been done experimentally and clinically with leukocyte extracts and, according to Zinsser,[1] there seems to be little question about their having a favorable influence both in experimental infections in animals and in the treatment of human cases, but there has been considerable difficulty in determining the reason for this influence.

Pettersson[2] found that leukocytes and leukocyte extracts possessed distinct bactericidal properties for various strains of proteus, but not for the cholera vibro and typhoid bacillus. Hiss[3] found that extracts of normal rabbit leukocytes had a distinct modifying and curative action in rabbit infections, due chiefly, he thought, to poison-neutralizing or destroying properties. Later Hiss[4] and Zinsser[5] found that distinct precipitates were formed when leukocyte extracts and bacterial extracts were mixed. Their observations tended to show that leukocytic substances exert only slight, if any, bactericidal action and do not of themselves inhibit to any considerable extent the development of bacteria; further that the extracts do not to any marked degree directly increase intraperitoneal phagocytosis. However, they did note that there seemed to be a more rapid accumulation of phagocytes in the peritoneal cavities of guinea-pigs infected with cholera spirilla when leukocytic extracts were injected with the bacteria. Later Zinsser states that "from subsequent experiments it is not impossible, in fact it seems probable, that the protective properties of the leukocyte extracts are attributable, in part at least, to their positively chemotactic effect."

Alexander[6] was the first to note that the injection of leukocyte extracts, prepared according to the method of Hiss and Zinsser, was followed by a marked leukocytosis within 24 hours. Archibald and Moore,[7] using extracts made directly from the blood of normal animals, found that the injection of such extracts into guinea-pigs produced a marked increase in the number of leukocytes in from 2-6 hours, the maximum being reached about 6 hours after the injection, the leukocytosis being more marked than that produced by the injection of nuclein. The increase in leukocytes was in the number of polymorphonuclear cells. Red-staining granules were observed in these cells, increasing in number for about six hours. Zinsser[1] later concludes that he is "inclined

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[1] Infection and Resistance, 1918.
[2] Centralbl. f. Bakteriol., I, O., 1905, 39, pp. 423 and 613.
[3] Jour. Med. Research, 1908, 19, p. 323.
[4] Ibid., p. 399.
[5] Ibid., p. 411.
[6] Brit. Med. Jour., 1911, 1, p. 355.
[7] Archiv. Int. Med., 1914, 14, p. 120.

to believe at present that the beneficial effects of leukocyte extracts are based on the same principles as that which determines the reactions following the injection of bacterial or any other protein." Zinsser and Tsen,[8] however, found that the injection of leukocytic extract does not arouse as vigorous a leukocytic response as does the injection of bacillary protein, but that on the other hand the leukocytic extract does not induce so severe a leukopenia.

In a previous article I [9] showed that the leukocytes in an aleuronat exudate in rabbits were more actively phagocytic than those in the blood, probably due to their being young cells. The following experiments were made to determine whether the activity of the new leukocytes produced by injection of leukocytic extracts were more actively phagocytic than normal cells.

The extracts were prepared according to the method of Hiss and Zinsser and also that of Archibald and Moore. Later, extracts were kindly furnished by Dr. John W. Anderson, director of the biological department of Squibb and Sons; these extracts were derived from the horse and were made according to the method of Hiss and Zinsser.

Hiss and Zinsser produced a double purulent exudate in the pleural cavities of rabbits by the injection of sterile aleuronat solution. This exudate was centrifuged quickly and the serum decanted. Distilled water in amount equal to that of the fluid poured off was added and the exudates thoroughly emulsified and allowed to stand for a few hours at 37 C. and then at icebox temperature until used. The cell residue as well as the supernatant fluid is injected.

Archibald and Moore used leukocytes directly from the blood of normal animals (horse, dog, or other domestic animal). They found that dog leukocytes produced the highest leukocytosis. I therefore used dog leukocytes. Blood was collected in a 1% sodium citrate solution, and 0.5 of 1% acetic acid was added. The mixture was centrifuged and the supernatant fluid discarded. The sediment was washed several times in salt solution and ground with quartz sand and neutralized. Distilled water was then added in the proportion of 4 parts of water to 1 part of sediment, and the mixture was kept at a temperature of 58 C. for 1 hour and then put in the icebox. The supernatant fluid was decanted and trikresol added.

The phagocytic activity of leukocytes is determined as follows: The leukocytes from rabbits injected with extract and from normal rabbits are collected in 2% sodium citrate solution, centrifuged and washed twice in normal salt solution to remove all trace of serum. To determine the activity of leukocytes it is essential to use suspensions containing approximately the same number of polymorphonuclear leukocytes. Such suspensions are obtained by counting the number of polymorphonuclear leukocytes in each and equalizing them with normal salt solution or by counting the number of cells in the circulating blood and collecting the same amount of blood from each rabbit and then

[8] Jour. Immunol., 1917, 2, p. 247.
[9] Trans. Chicago Path. Soc., 1911, 8, p. 208.

equalizing according to the count. The same serum is used to provide the necessary opsonin in the test of the two kinds of leukocytes. Any organism which is not spontaneously phagocytable may be used, but a virulent organism which is not opsonized by normal serum for normal leukocytes is not suitable. Pneumococci and Strep. viridans were used in my experiments. Equal parts of serum, leukocytic suspension and bacterial suspension, are mixed in bent capillary pipets and incubated 25 minutes. The mixtures are smeared on glass slides and stained. The phagocytic activity of the leukocytes is determined by comparing the number of bacteria taken up by the two sets of leukocytes (cytophagic index). Fifty cells are counted on each slide.

From 2 to 10 c c of the leukocytic extract were injected subcutaneously in 5 rabbits. Two rabbits were injected with rabbit exudate extract, one with dog leukocyte extract, the other rabbits with extracts of horse leukocytes. Leukocyte counts were made before and every two or three hours for six hours after the injection, and then daily until the count returned to normal. The activity of the leukocyte was estimated from time to time. A leukopenia was not observed following the injections, but no counts were made before two hours after the injection of the extract.

The highest leukocyte count as a rule was reached about six hours after the injection, the horse exudate causing a somewhat slower rise than the other exudates. The leukocytosis lasted from one to four days, and the increase was in the number of the polymorphonuclear cells. No change in their staining reaction was observed. The average number of leukocytes in the normal control rabbit was 8,000, and the average highest leukocyte count in the injected rabbit was 15,900. During the height of the leukocytosis the polymorphonuclear cells were found to be from two to four times more actively phagocytic than normal. Repeated injections of extract caused an increase in the number and activity of the leukocytes, but not as a rule greater than that produced by the first injection.

Two rabbits were injected intravenously, one with 7, the other with 3 c c of extract of horse leukocytes. The number of leukocytes in the rabbit receiving 7 c c began to rise in 15 minutes, increasing 8,000 in 30 minutes and 13,200 in one hour. The number returned to normal two hours after the injection. During the height of the leukocytosis the polymorphonuclear cells were three times more active than normal cells. The second rabbit received 3 c c and this produced a slight fall in the number of leukocytes for 30 minutes, followed by a

rise of 5,000 in one hour and a rise of 21,500 in two hours. The number of leukocytes returned to normal three hours after the injection. During the height of the leukocytosis the polymorphonuclear cells were eight times more actively phagocytic than normal.

I have not tried to determine whether there is an increased activity of the cells in leukocytosis produced by other proteins.

I have shown [10] that the leukocytes in the leukopenia of measles and influenza are considerably less actively phagocytic than normal cells. Hence it seemed desirable to determine whether the leukocytes in leukopenia could be stimulated to greater activity by the injection of leukocytic extract. Leonard [11] has found that the administration of leukocyte extract in measles and influenza results in an increase in the number of leukocytes. As I was unable to study the activity of leukocytic extract in measles and influenza, leukopenia was produced in rabbits by the subcutaneous injection of benzene and olive oil (1 c c per kilo of weight). After three injections a distinct decrease in the number of leukocytes occurred, and one rabbit now received three daily subcutaneous injections of 10 c c of extract of horse leukocytes, but without in any way affecting the leukopenia. Another rabbit received simultaneously benzene and leukocytic extract together on three successive days, but the leukopenia was not prevented. The leukopenia produced by benzene is, of course, not comparable with that produced by infection, and observations must be made in cases of infection with leukopenia to determine definitely whether leukocytic extracts can then increase the phagocytic activity of leukocytes.

SUMMARY

The results of the experiments show that the subcutaneous injection of leukocytic extract in rabbits produces an appreciable increase in the number of leukocytes in the circulating blood lasting from one to four days. The leukocytes set free by the extract possess considerably more phagocytic power than normal leukocytes. While the intravenous injection of leukocytic extract produces a more rapid rise in the number and activity of the leukocytes, the duration is shorter than that produced by subcutaneous injection. Leukocytic extract appears to exert no influence on the leukopenia produced by benzene.

[10] Jour. Infect. Dis., 1912, 11, p. 474; Jour. Am. Med. Assn., 1918, 71, p. 1733.
[11] Jour. of Med. Soc. N. J., 1919, 16, p. 354.